Tropine-labeled Atropine Metabolism in Man

N-$C^{14}H_3$-Atropine. S.C. Kalser and P.L. McLain

Because of the extremely low doses of atropine which man can tolerate, it has been difficult to obtain information by chemical means of the manner in which atropine is metabolized in the human. The scarcity of reliable metabolic and excretory data stems largely from the inadequate sensitivity and specificity of conventional methods of analysis.

The first and only study to date preceding this one, that attempts to elucidate the fate of atropine in man is that of Gosselin et al (1960) in which a labelled atropine specifically tagged in the tropic acid moiety was used. Two male subjects were studied, and the conclusions reached were that, in contrast to data previously obtained in rodents (Gosselin et al, 1955; Kalser et al, 1957; Gabourel and Gosselin, 1958) atropine showed little modification in its tropic acid ring and that the main metabolites were apparently compounds in which the tropine ring were modified.

Because the limited observations in man suggested that the tropine part of the atropine molecule might be modified in man, the need for specifically labeled atropine with a tracer in the tropine ring was apparent. The first of a series of C^{14}-ring labeled atropines to be synthesized by G.C. Schmidt and T.E.Eling, was an N-Me^{14}-atropine. This compound has now been observed in one normal subject. The excretion of the C^{14} in the urine and expired air, and the disappearance of the C^{14} from the blood after an intramuscular injection of 2 mg. is presented in this report. Chromatographic analysis of urinary components is incomplete at this time, but preliminary data suggests that most of the label is present in the urine as unchanged atropine and as its hydrolyis product, tropine. A total of 88% of the injected dose was collected in the urine in 48 hours. A unique finding was the occurence of C^{14} in the expired air, indicative of a demethylation being one of the metabolic pathways for atropine in man. Approximately 3 % of the injected dose was recovered in the expired air in the 3 hours during which samples were taken. Blood levels were extremely low, indicative of avid tissue binding of the drug, but did seem to reach a maximum at 30 minutes after injection, a time at which maximum tachycardia was also observed. Expired air $C^{14}O_2$ showed a maximum at 75 minutes, while the peak urinary concentration occured at 3 hours.

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Methods:

Subject: A white, 38 year old, healthy female weighing 70.9 kg. was injected with 2.0 mg of N-$C^{14}H_3$-atropine having a specific activity of 3.367 uc/mg. and having a radiopurity of 94%. The one contaminating radioimpurity is chromatographically similar to tropine, a precursor in the synthesis. The C^{14}-atropine was prepared as a solution of the sulfate salt in isotonic saline and buffered with phosphate buffer to a pH of 5.75 prior to sterilization by autoclaving. The injected solution contained 2 mg/ml of the free base.

Experimental plan: Electrocardiographic limb leads were attached and an indwelling intravenous needle connected to a 3-way stopcock were attached to the subject prior to the i.m. injection. Blood samples (2 ml dispensed into test tubes containing 0.1 ml of 1:1000 heparin) and EKG records were taken every 5 minutes for the first 60 minutes. Aliquots of expired air, consisting of a timed collection of a known volume into a spirometer, were taken every 15 minutes for 2 hours and at 30 minute intervals for the third hour. Two-liter aliquots of the expired air sample was passed through 10 ml of Hyamine as a CO_2 absorber in a period of 4 to 9 minutes. The $Ba(OH)_2$ indicator suggests that there may have been a slight loss of the CO_2 sample and that therefore the results may be slightly in error on the low side. Urine samples were collected hourly up to 8 hours, then at 12, 24, 36 and 48 hours. Aliquots of 0.8 ml were counted in a cellosolve counting solution and compared with the administered standard which was similarly prepared. Four ml of the Hyamine solution contained the CO_2 of the expired air was counted with 15 ml of the cellosolve solution. Paper chromatograms were prepared from the original urine and counted with a strip scanner after chromatographic development in an n-butanol:acetic acid:water (86:14:32.5) solvent system. Further processing of the urine (concentration by freeze drying followed by hydrolysis and cleavage of glucuronides) is presently in progress.

Results: **Tropine-labeled Atropine Metabolism in Man; N-$C^{14}H_3$-atropine**

A. C^{14} in Urine: Injected dose = 1.0924 x 10^7 dpm

Time	dpm/0.8 ml	ml urine	% inj. dose	Σ % inj. dose	% inj. dose/10 min
1 hr.	12,981	41	6.090	6.090	1.015
2 hr.	14.708	82	16.830	22.920	2 805
3 hr.	5,000	340	19.454	42.374	3.242
4 hr.	33 76	355	13.716	56.090	2.286
5 hr.	3,562	238	9.700	65.790	1.617
6 hr.	5,990	78	5.346	71.136	0.891
7 hr.	6,945	47	3.735	74.871	0.622
8 hr.	6,244	29	2.072	76.943	0.345
12 hr.	4,996	94	5.373	82.316	0.224
24 hr.	702	550	4.418	86.734	0.0614
36 hr.	173.8	435	0.865	87.599	0.0120
48 hr.	45.0	1285	0.662	88.261	0.0092

B. C^{14} in Expired Air: Injected dose = 1.04557 x 10^7dpm

Time	dpm/0.8 L.	L/period(av)	% inj. dose	Minute vol (av.) = 9.99 ± 0.625 L
15 min.	45.7	148.5	0.0837	
30 min.	87.1	"	0.1562	
45 min.	149.2	"	0.2675	
60 min.	189.4	"	0.3394	
75 min.	212.4	"	0.3807	
90 min.	163.4	"	0.2929	
105 min.	160.4	"	0.2875	
120 min.	156.7	"	0.2809	
150 min.	132.0	297.0	0.4687	
180 min.	132.2	"	0.4695	
			Σ = 3.0270	

C. C^{14} in Blood: Injected dose = 1.0929 x 10^7dpm

Time	dpm/ml	Hct.	Pulse (min) pre-injection = 95	
5 min.	88.8	39.5	61	
10 min.	114.4	38.7	98	
15 min.	156.1	39.4	116	
20 min.	142.6	39.4	124	
25 min.	142.5	39.0	124	
30 min.	161.4	39.0	128	
35 min.	146.0	39.4	124	
40 min.	142.5	39.4	122	
45 min.	152.0	39.2	124	
50 min.	158.2	39.4	120	
55 min.	136.4	39.5	118	
60 min.	137.1	39.8	120	
240 min.			90	Not dry
90 min			120	

DISCUSSION:

The urinary excretion of the C^{14} label, after the i.m. administration of tropine labelled atropine, is extremely rapid. As is evident in Figure 1, the timed excretion reaches a maximum at 3 hours and then declines in an exponential manner, showing at least 3 rate constants. The fastest disappearance rate has a biological half-time of 1½ hours and may be indicative of the renal clearance of the drug uncomplicated by the slower excretion (half time of 6 hours) which may be a reflection of the enterohepatic reabsorption with final excretion. The third $t_{\frac{1}{2}}$ is 30 hours, and may represent the rate of metabolism of the drug.

Table 1, Part A, shows the percent of the injected dose which is excreted at each time period as well as the cumulative percent of the injected dose which has been excreted. By 4 hours, over 50% of the drug has been committed to the urine. This figure agrees extremely well with a previous study reported by Gosselin, Gabourel and Hills (1960) using a C^{14}-tropic acid labelled atropine in a 73 year old post-prostatectomy and vasectomy patient having an indwelling Foley cathethr. The 8, 24 and 48 hour values from this study and from our study are also in remarkably close agreement; 88% of the administered dose being accounted for in both cases. However, the second subject reported in the same study, a healthy 45 year old male, showed a much slower initial excretion (only 18% in 4 hours), but by 24 hours the cumulative excretion had also reached 85%.

A unique finding for the studies performed in man is the detection of small amounts of $C^{14}O_2$ in the expired air. Since only timed aliquots of the total expired air could be sampled, calculations for the total amount excreted by this pathway were made by considering that the specific activity of the sample collected was representation of the concentration over the entire sampling interval, and calculating the amount from the known average minute volume. As can be observed in Table 1, Part B, the C^{14} concentration in the expired air reached a maximum at an earlier time than did the urine, maximum activity being seen at 75 minutes compared with 180 minutes for urine. The total percent of the injected dose collected during the 3 hour period was about 3%, and there is some indication that perhaps an additional 2-3% could have been realized had the sampling been continued In an early study with randomly labelled (biosynthetically prepared) C^{14}-atropine,

Evertsbusch and Geiling (1953) did find a small amount of $C^{14}O_2$ (0.8% in 24 hours) present in the expired air from the mouse, and at least part of this $C^{14}O_2$ probably originated with the N-methyl group. With the tropic acid labeled atropine (Gosselin et al, 1960), no C^{14} was detected in the expired air in man, nor in the mouse (Gosselin et al, 1955).

Tissue binding of atropine is so marked that high blood levels of the drug are not seen, even in the very early blood samples (see Table 1, part C). If the drug were confined to the vascular compartment, then at zero time the calculated blood concentration would be 2200 dpm/ml blood. This compares with the maximum of 161 dpm/ml found experimentally. At even distribution throughout the body, the concentration of C^{14} would be 154 dpm/gm., a figure in much closer agreement with the values seen for the blood and indicative of an extremely rapid egress from the blood. The rapid disappearance of atropine from the blood was also seen in studies on the isolated, perfused rat liver (Kalser et al, 1965) in which a $t_{\frac{1}{2}}$ of 6 minutes was seen when the liver alone was responsible for its clearance from the blood.

The initial marked bradycardia observed on the EKG record taken 5 minutes after the i.m. administration of the drug (Table 1, part C) attests to the extremely rapid absorption of the drug. This effect, which appears to be a response seen only at low concentrations of atropine, and then probably only in man, quickly reverses to the more commonly seen tachycardia as the blood level becomes higher. The tachycardia reaches a maximum at 30 minutes, the same period at which the maximum blood concentration is seen. By 4 hours, a time at which the subject reported an adequate flow of saliva was again evident, the tachycardia had subsided. By this time, 50% of the drug had already been excreted into the urine.

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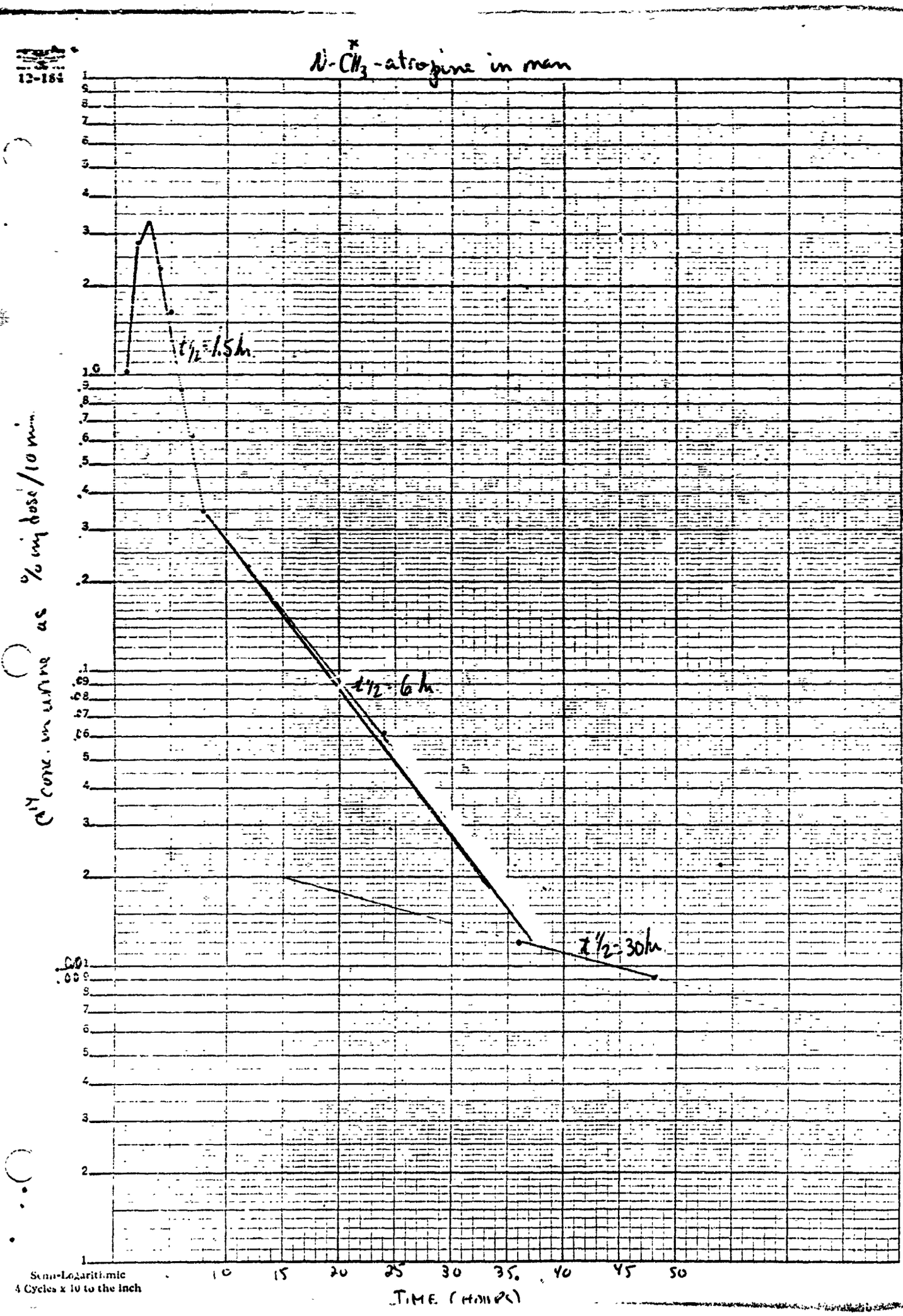
N-CH3*-atropine in man
t1/2=1.5h
t1/2=6h
t1/2=30h
Conc. in urine as % inj dose/10 min
TIME (HOURS)
Semi-Logarithmic
4 Cycles x 10 to the inch